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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,282,007,** on September 9, 1999, by **QUEEN ELIZABETH II, HEALT SCIENCE CENTRE,** assignee of Ivar Mendez, for "Neural Transplantation Delivery System".

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ABSTRACT

A device and method for neural transplantation in the human brain comprising a microinjector, transplantation cannula and bullet guide is disclosed. The microinjector is designed to connect to the proximal end of a syringe barrel and plunger while the transplantation cannula interfaces with the distal end of the syringe barrel. In combination, the microinjector and transplantation cannula permit the delivery of multiple cell grafts in a three-dimensional array using a unique spiral technique. The bullet guide, which is attachable to a commercially available stereotactic frame, is a multiple channel adapter that functions as a mechanical guiding system for the transplantation cannula and permits plural, spaced deployment of the cannula without adjusting or disturbing the frame.

NEURAL TRANSPLANTATION DELIVERY SYSTEM

FIELD OF INVENTION

The present invention relates to a device and method for neural transplantation in the human brain comprising a microinjector, transplantation cannula and bullet guide. The microinjector and transplantation cannula are adapted to connect to opposite ends of a syringe in a simple manner. The bullet guide, comprised of mutually spaced top and bottom portions, is mounted to a stereotactic frame and functions as a mechanical guiding system for the cannula. In combination, the invention provides a simple, reliable and safe system for delivering and maximizing the number of cell graft deposits to the host brain with minimal trauma using a unique spiral technique.

BACKGROUND OF THE INVENTION

Neural transplantation of fetal ventral mesencephalic (VM) tissue has been studied for the past two decades as a potential surgical strategy for the treatment of Parkinson's disease (PD). Clinical trials in Parkinsonian patients have been conducted in several centres worldwide with more than 200 patients receiving fetal transplants into the striatum (Mehta et al., *Can. J. Neurol. Sci.*, 24, pp. 292-301, 1997; Olanow et al., *TINS*, 19, pp. 102-109, 1996; Rehncrona et al., *Adv. Tech. Stand. Neurosurg.*, 23, pp. 3-46, 1997; Tabbal et al. *Curr. Opin. Neurol.*, 11, pp. 341-349, 1998). Survival of the grafts has been documented with positron emission tomography (PET) scanning (Freeman et al., *Ann. Neurol.*, 38, pp. 379-388, 1995; Remy et al., *Ann. Neurol.*, 38, pp. 580-588, 1995; Wenning et al., *Ann. Neurol.*, 42, pp. 95-107, 1997) and postmortem studies (Kordower, et al., *N. Engl. J. Med.*, 332, pp. 1118-1124, 1995). Although the results of these trials have been promising,(Hauser et al., *Arch. Neurol.*, 56, pp. 179-187,

1999; Wenning et al., *Ann. Neurol.*, 42, pp. 95-107, 1997) clinical efficacy has not reached the stage for neural transplantation to become a routine therapeutic procedure for PD. Implantation trauma, which decreases graft survival, and inadequate reinnervation of the host striatum due to suboptimal distribution of graft deposits are considered detrimental factors in achieving optimal clinical efficacy. Decreased implantation trauma and a more complete reinnervation of the dopamine-depleted striatum have been achieved in animal models of PD by decreasing the size of the implantation cannula and increasing the number of deposits of fetal dopaminergic cells (Nikkhah et al., *J. Neurosci.*, 15(5), pp. 3548-3561, 1995; Nikkhah et al., *Neurology*, 63, pp. 57-72, 1994). These modifications to the implantation technique have produced improvements in host reinnervation and functional recovery in the rodent model of PD (Nikkhah et al., *J. Neurosci.*, 15(5), pp. 3548-3561, 1995; Nikkhah et al., *Neurology*, 63, pp. 57-72, 1994).

The use of neural transplantation to treat neurological conditions such as PD has the potential to be an important therapeutic strategy in the near future. There is strong evidence of long-term survival of transplanted dopaminergic neurons (Kordower et al., N. Engl. J. Med., 332, pp. 1118-1124, 1995) and clinical results are promising (Hauser et al., Arch. Neurol., 56, pp. 179-187, 1999; Wenning et al, Ann. Neurol., 42, pp. 95-107, 1997). Transplantation in patients with Huntington's disease has also been reported (Kopyov et al., Cell Transplantation for Neurological Disorders, Humana Press, pp. 95-134, 1998) and porcine xenografts are being studied in clinical trials (Deacon et al., Nature Medicine, 3, pp. 350-353, 1997; Isacson et al., Nature Medicine, 1(11), pp. 1189-1194, 1995; Schumacher et al., Nature Medicine, 3, pp. 474-475, 1997). A great deal of experimental work in animals is being conducted for novel cell types as an alternative source to human fetal

tissue for neural transplantation. This research may expand the use of reconstructive strategies in the future (Borlongan et al., Exp. Neurol., 149, pp. 310-321, 1998; Fitoussi et al., Neuroscience, 85, pp. 405-413, 1998; Svendsen et al., Exp. Neurol., 137, pp. 376-388, 1996).

SUMMARY OF THE INVENTION

An object of the invention is to provide a delivery system for neural transplantation grafts with minimal trauma to the host brain.

Another object of the invention is to provide a delivery system including a microinjector and neural transplantation cannula that can be easily incorporated with a syringe to facilitate reliable and safe neural transplantation of cell grafts to the human brain, particularly in the treatment of Parkinson's disease.

A further object of the invention is to provide a device including a microinjector and neural transplantation cannula which, in combination with a syringe, are designed to decrease implantation trauma and maximize the number of graft deposits per injection using a unique spiral technique.

Still another object of the invention is to provide a bullet guide which, when mounted to a stereotactic frame, functions as a mechanical guiding system for the transplantation cannula thereby permitting multiple access of the cannula without adjusting or disturbing the frame.

According to one aspect of the invention there is provided a neural transplantation device which comprises:

(a) a syringe including a syringe barrel and plunger;

(b) a microinjector adapted for connection to the proximal end of the syringe barrel and for cooperation with the

plunger for effecting incremental depression and retraction of the plunger; and

(c) a cannula adapted for connection to the distal end of the syringe barrel, said cannula having a blunt closed lower end and being formed with a pair of offset holes on opposite sides of the cannula for fluid delivery;

(d) whereby upon placement of the cannula in a targeted cerebral site, operation of the microinjector to effect incremental depression of the plunger results in metered injection of fluid from the syringe barrel through the cannula holes to a targeted site.

A particular embodiment provides a microinjector and neural transplantation cannula for use in combination with a syringe, comprising:

(a) a longitudinal cylindrical sleeve which extends into a cylindrical barrel of larger diameter at the distal end thereof;

(b) a guide nut adjustably rotated within the cylindrical barrel and adapted to cooperate with the proximal end of a syringe barrel, the drive nut being rotatably mounted on the cylindrical sleeve;

(c) a plunger driver rotatably mounted on the cylindrical sleeve and cooperating with both the drive nut and a syringe plunger; and

(d) a hollow cannula which is closed at its extreme distal end and has a pair of holes near the distal end that are diametrically opposed and slightly offset to each other;

(e) whereby placement of the transplantation cannula in contact with a targeted cerebral site followed by rotation of the drive nut renders a downward axial force to the plunger of the syringe thereby aspirating the fluid contents of the syringe barrel from the holes to effect delivery of an injection; followed by rotation of the guide nut in the opposite direction to move the syringe in an upward vertical direction to allow repositioning of the

cannula for subsequent delivery of injection fluid; and rotating the drive nut and guide nut in a repeated sequential manner to distribute multiple portions of injection fluid along a single trajectory in a three-dimensional spiral array at predetermined injection sites.

Another aspect of the invention provides a bullet guide for use in combination with a stereotactic frame which functions as a mechanical guiding system for the transplantation cannula, the bullet guide comprising:

(a) a top portion comprising a hollow cylindrical element having a closed end with an array of equidistantly spaced holes sized to accommodate the insertion of the transplantation cannula; and

(b) a bottom portion comprising a hollow cylindrical element of the same diameter as the top portion but having a longer longitudinal axis; said portion being closed at both ends and each end having an array of equidistantly spaced holes sized to accommodate the insertion of the transplantation cannula;

(c) whereby the top portion and bottom portion are mounted in spaced coaxial alignment, in a stereotactic frame with the respective arrays of holes in mutual alignment to guide deployment of the transplantation cannula through an aligned pair of said holes to a predetermined cerebral target.

Thus, the present invention affords a microinjector and transplantation cannula adapted and designed for use, for instance, with a 50 µl Hamilton syringe. The Hamilton syringe comprises a syringe barrel, which receives fluid contents, and a rod-like plunger for expelling the fluid contents from the barrel. In the assembled relationship, the microinjector and cannula create a secure and cooperative attachment to the extreme proximal and distal ends, respectively, of a Hamilton syringe, such that all

the components are coaxially aligned to one another.

The microinjector essentially comprises a longitudinal cylindrical sleeve which is threaded on its exterior surface and extends abruptly into a plunger guide at its distal end that has a larger diameter than the sleeve. The exterior surface of the plunger guide is uniform and its internal diameter is sized to fit and cooperate with the peripheral shoulder of the barrel of a syringe. The inner wall of the plunger guide is threaded to match and interface with a guide nut which is adjustably rotated inside the barrel. The guide nut is a small hollow cylindrical spool with a collar at its extreme distal end that acts as a lower boundary stop to limit its position inside the plunger guide when fully wound. In turn, the guide nut is designed to securely interface with the syringe immediately beneath the peripheral shoulder located at the extreme proximal end of the barrel. Accordingly, attaching the guide nut to the barrel converts the syringe to an adjustably rotated device that can easily be wound inside the plunger guide. Therefore, rotating the guide nut in either a clockwise or counter-clockwise direction simultaneously rotates the syringe in the same direction. Depending on the direction of rotation, this operation ultimately translates into either an upward or downward vertical movement of the syringe. Therefore, the vertical distance in which the syringe moves by rotation of the guide nut is a function of the length and diameter of the plunger guide and guide nut.

Mounted at the proximal end of the cylindrical sleeve is a threaded drive nut engaged with a threaded plunger driver which are both adjustably rotated in either a clockwise or counter-clockwise direction. As a result of their connection, rotating one element moves the other element simultaneously. The plunger driver is engaged with the proximal end of a syringe plunger such that when the driver

is rotated, the movement of the plunger is controlled in either an upward or downward direction along a longitudinal axis parallel to the syringe. Therefore, during neural transplantation, rotation of the plunger driver results in delivery of a desired volume of cell suspension contained within the syringe barrel. The microinjector is advantageously manufactured from acetal nylon and ionized aluminum.

The transplantation cannula of the present invention advantageously comprises a long narrow needle provided with a standard Luer lock at its proximal end. The Luer lock allows the cannula to be readily attached to and in fluid connection with the contents of the syringe, and then easily removed following use. The tip of the cannula at the extreme distal end is closed and blunt and its outer surface is polished and rounded, for instance in a hemispherical shape, to minimize trauma to neural tissue during insertion. Located near the tip of the cannula are a pair of port holes to allow egress of cells during aspiration of the syringe. The port holes are advantageously diametrically opposed and slightly offset to each another. This arrangement minimizes brain trauma, while maximizing cell graft deposits. The use of a pair of holes is important since a larger number of holes would tend to increase the risk of trauma and possible damage to neural tissue. Likewise, the positioning of the holes on opposite sides of the cannula in an offset arrangement is important for obtaining adequate delivery and distribution of cell graft deposits. The transplantation cannula is advantageously manufactured from stainless steel.

The bullet guide, which comprises both a top portion and a bottom portion, is mounted to a stereotactic frame and functions as a mechanical guiding system for the transplantation cannula. The top portion of the bullet guide, being the stop bullet, is a hollow cylindrical tube

which is closed at its proximal end and circumscribed by a peripheral collar. The surface of the closed end embodies a square grid, preferably consisting of nine holes equidistantly spaced apart and sized to accommodate the diameter of the transplantation cannula. The bottom portion, being the guide bullet, is a hollow cylindrical tube of similar diameter to the stop bullet but with a longer longitudinal axis. The guide bullet is closed at both the proximal and distal ends and the surface of each end has a square grid identical to the stop bullet to accommodate the insertion of the transplantation cannula. In addition, the guide bullet is circumscribed by a peripheral collar at its extreme proximal end and has an inwardly tapered portion with a flat surface at its extreme distal end. The peripheral collar of each of the guide bullet and stop bullet contains an indexing groove formed of a particular dimension and shape to allow both portions to selectively interface and cooperate with a commercial stereotactic frame when mounted. The positioning of the indexing groove ensures that when the stop bullet and guide bullet are mounted, their grids will be coaxially aligned one above the other thereby allowing the transplantation cannula to be precisely guided and inserted at a predetermined cerebral target. Both the stop bullet and guide bullet are advantageously manufactured from stainless steel and each component can preferably be disassembled into four separate parts to allow for effective cleaning and sterilization.

Prior to operation of the neural transplantation device, the microinjector, syringe and transplantation cannula are mounted in the stereotactic frame, positioned at a predetermined location and oriented at the cerebral target site using the guide bullet to direct the cannula. The desired cerebral target site is generally identified by a diagnostic imaging technique (e.g. magnetic resonance imaging, computerized tomography, ultrasound, or the like).

During the initial stage of operation, the plunger of the syringe is in a foremost upward position and the syringe barrel with attached guide nut is in an unwound position inside the plunger guide. When an injection is to be administered, the plunger driver is rotated, thereby advancing the syringe plunger in a downward vertical direction through the syringe barrel. A specific volume of the cell suspension is subsequently aspirated and deposited through the port holes of the transplantation cannula at the target site. Prior to making a second injection and deposit of the cell suspension, the guide nut is rotated 90° in a clockwise direction thereby incrementally retracting the syringe and cannula in an upward vertical direction at a predetermined distance away from the first target site. Aspiration and delivery of a second volume of cell suspension is made by repeating the operation involving rotation of the plunger driver. Sequential repetition of the steps involving rotation of the plunger driver and guide nut to deliver the contents of the syringe and reposition the cannula, respectively, allows several injections to be made per single trajectory thereby distributing the cells in a three-dimensional spiral array within the brain tissue. Consequently, control of delivery of the cell suspension, location of the port holes of the transplantation cannula and the distance of syringe movement enable the user to employ the microinjector device with accuracy and precision at a cerebral target site.

Additional cell deposits at different trajectories are made by removing the microinjector device from its operative position, governed by the square grids of the bullet guide, and then reinserting the transplantation cannula of the microinjector through another specified landmark within the grid.

Thus, the invention affords a simple and reliable method to deposit graft material into the brain using a

transplantation cannula and microinjector system easily adaptable to any stereotactic frame. The two-hole design of the cannula tip has been validated by animal experiments which demonstrated the ability of the cannula to deliver two distinct graft deposits per injection. This design allows for graft deposits to be placed no more than 2 mm apart from each other. This distance is close enough for the grafts to become confluent since fibre outgrowth has been shown to extend 2 to 7 mm into the host tissue in human transplantations (Kordower et al., n. Engl. J. Med., 332, pp. 1118-1124, 1995). In the grafted rats, the cannula tract facilitated the connection of the two graft deposits. Proper distribution of graft deposits to facilitate confluency in all three dimensions may improve host reinnervation and clinical outcome (Freed et al., N. Engl. J. Med., 327(22), pp. 1549-1555, 1992; Freeman et al., Ann. Neurol., 38, pp. 379-388, 1995).

Implantation trauma is known to be detrimental to graft survival (Nikkah et al., Brain res., 633, pp. 133-143, 1994; Nikkah et al., Neurology, 63, pp. 57-72, 1994) and applicant's animal experiments showed excellent graft survival with no significant trauma to transplanted rat striatum, which is an indication of the atraumatic nature of the cannula design. This observation in the experimental model correlates well with the absence of hemorrhage or tissue damage on the 24 hour post-operative MRI scans of transplanted patients. There was also an increase in fluorodopa uptake on PET imaging after transplantation. At present, the only valid method to assess graft survival in vivo is by measuring fluorodopa with PET scans. Fluorodopa is an analog of levodopa, which is taken over the blood-brain-barrier, decarboxylated and stored in the nigrostriatal dopaminergic terminals. Correlation of graft survival and fluorodopa PET scans has been made by a postmortem examination of a patient transplanted with fetal VM tissue 18 months before death of

causes unrelated to the transplant procedure (Kordower et al., N. Engl. J. Med., 332, pp. 1118-1124, 1995).

The cannula is designed to optimize host reinnervation by maximizing the number of deposits per pass. Increasing the density of reinnervation per pass may lead to a reduction in the number of passes through the brain and decrease the chance of hemorrhagic complications. The cannula may be used with cell suspensions that are not completely dissociated and contain "chunks" of fetal VM and no problem has been encountered with the aspiration or delivery of this "chunky" cell preparation. Delivery of solid "cores" of fetal VM have been previously described in the literature using a "double-cannula system" (Breeze et al., Neurosurgery, 36, pp. 1044-1048, 1995).

Accordingly, the present invention provides a simple, safe and reliable neural transplantation delivery system. As neural transplantation evolves and the clinical efficacy of this strategy for the treatment of neurological conditions is established, the ability to deliver viable grafts with minimal trauma may play an important role in neurosurgery.

The experimental and clinical experience with the use of a neural transplantation cannula and microinjector system specifically designed to decrease implantation trauma and maximize the number of graft deposits per injection is also provided. Animal studies conducted using the rat model of PD during the experimental stage of this study demonstrated excellent graft survival with minimal trauma to the brain. Following this experimental stage, the cannula and microinjector system were employed in eight Parkinsonian patients enrolled in the Halifax Neural Transplantation Program who received bilateral putaminal transplants of fetal VM tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following description, the invention will be explained in detail with the aid of the accompanying drawings which illustrate preferred embodiments of the present invention and in which:

Figure 1 illustrates a cross-section of a microinjector and neural transplantation cannula fitted to a syringe;

Figure 2 illustrates a perspective view of a stop bullet and guide bullet in cooperation with the transplantation cannula of Figure 1;

Figure 3 is an axial view of the stop and guide bullets;

Figure 4 shows the microinjector and neural transplantation cannula fitted to a stereotactic frame;

Figures 5A and 5B illustrate front and top views, respectively, of a sequence of graft deposits using the neural transplantation cannula;

Figure 6 is a photomicrograph of a coronal section of a rat striatum immunostained with tyrosine hydroxylase (TH) to visualize dopaminergic neurons;

Figure 7 shows high power photomicrographs of the section shown in Figure 6;

Figure 8 is a MRI scan (inversion recovery) 24 hours after surgery showing four graft deposits in the right putamen; and

Figure 9 provides fluorodopa PET scans of a patient transplanted using the microinjector and transplantation cannula.

DETAILED DESCRIPTION

As illustrated in Figure 1, an embodiment of the present invention affords a microinjector 1 and transplantation cannula 2 adapted and designed for use with a 50 µl Hamilton syringe 3.

The microinjector 1 essentially comprises a longitudinal cylindrical sleeve 4 which is threaded on its exterior surface and extends abruptly into a plunger guide 5 at its distal end that has a larger diameter than the sleeve 4. The exterior surface of the plunger guide 5 is uniform and its internal diameter is sized to fit and cooperate with the peripheral shoulder 6 of the barrel 7 of a Hamilton syringe 3. The inner wall of the plunger guide 5 is threaded to match and interface with a guide nut 8 which is adjustably rotated inside the barrel. The guide nut 8 is a small hollow cylindrical spool with a collar 9 at its extreme distal end that acts as lower boundary stop to limit its position inside the plunger guide 5 when fully wound inside. In turn, the guide nut 8 is designed to securely interface with the Hamilton syringe 3 immediately beneath the peripheral shoulder 6 located at the extreme proximal end of the barrel 7. Accordingly, attaching the guide nut 8 to the barrel 7 coverts the syringe 3 to an adjustably rotated device that can easily be wound inside the plunger guide 5. Therefore, rotating the guide nut 8 in either a clockwise or counter-clockwise direction simultaneously rotates the syringe 3 in the same direction. Depending on the direction of rotation, this operation ultimately translates into either an upward or downward vertical movement of the syringe 3. Therefore, the vertical distance in which the syringe 3 moves by rotation of the guide nut 8 is a function of the length and diameter of the plunger guide 5 and guide nut 8.

Mounted at the proximal end of the cylindrical sleeve 4 is

a threaded drive nut 10 engaged with a threaded plunger driver 11 which are both adjustably rotated in either a clockwise or counter-clockwise direction. As a result of their connection, rotating either the drive nut 10 or plunger driver 11 moves the other element simultaneously. The plunger driver 11 is engaged with the proximal end of a syringe plunger 12 such that when the driver 11 is rotated, the movement of the plunger 12 is controlled in either an upward or downward direction along a longitudinal axis parallel to the syringe 3. Therefore, during neural transplantation, rotation of the plunger driver 11 results in delivery of a desired volume of cell suspension contained within the syringe barrel 7.

The transplantation cannula 2 is a long narrow needle provided with a standard Luer lock 13 at its proximal end. The Luer lock 13 allows the cannula 2 to be readily attached to and in fluid connection with the contents of the syringe 3, and then easily removed following use. The tip of the cannula 2 at the extreme distal end 14 is closed and its outer surface has been rounded and polished in a semi-spherical shape to minimize trauma to neural tissue upon insertion. Located near the tip of the cannula are a pair of holes, 15A and 15B, to allow egress of cells during aspiration of the syringe 3 and which are diametrically opposed and slightly offset to one another. In the embodiment shown, hole 15B is located 1.0 mm from the cannula tip 14 and hole 15A is offset from hole 15B by a distance of 2.0 mm.

The bullet guide 16, illustrated in Figure 2, comprises both a top portion and a bottom portion that are mounted to a stereotactic frame and function as a mechanical guiding system for the transplantation cannula 2. The top portion of the bullet guide 16, comprises a stop bullet 17 and is a hollow cylindrical tube which is closed at its proximal end and circumscribed by a peripheral collar 18. The surface

of the closed end embodies a square grid 19 consisting of nine holes 19A spaced an equidistance apart to one another and sized to accommodate the diameter of the transplantation cannula 2. The bottom portion of the guide 16 is a guide bullet 20, which is a hollow cylindrical tube of similar diameter to the stop bullet 17 but with a longer longitudinal axis. The guide bullet 20 is closed at both the proximal and distal ends and the surface of each end has an identical square grid 21 of holes 21A and 21B, respectively, to the grid 19 of holes 19A of the stop bullet 17 to accommodate the insertion of the transplantation cannula 2. Furthermore, the guide bullet 20 is circumscribed by a peripheral collar 22 at its extreme proximal end and has an inwardly bevelled portion 23 with a flat surface at its extreme distal end.

Figure 3 provides an axial view of the bullet guide 16 showing both the stop bullet 17 and guide bullet 20, and illustrating the square grids, 19 and 21, each consisting of nine holes, 19A, 21A and 21B, respectively, located equidistantly from one another. Also illustrated is the partitioning of both the stop bullet 17 and guide bullet 20 such that each component can be disassembled into four separate parts to allow for effective cleaning and sterilization. Various different interlocking or interfitting arrangements of parts are also contemplated. Peripheral collars 18 and 22 of the stop bullet 17 and guide bullet 20, respectively, contain indexing grooves, 24A and 24B, formed of a particular dimension and shape to allow both portions to selectively interface and cooperate with a commercial stereotactic frame when mounted. The positioning of the indexing grooves 24A and 24B ensures that when the stop bullet 17 and guide bullet 20 are mounted, their grids, 19 and 21, with holes 19A, 21A and 21B, will be coaxially aligned one above the other thereby allowing the transplantation cannula to be precisely guided and inserted at a predetermined cerebral target

The nine holes **19A** have been individually labelled **A, B, C, D, E, F, G, H** and **I**, and every hole **19A** in the stop bullet **17** lines up with holes **21A** and **21B** of the guide bullet **20**. Thus, when the bullets **17** and **20** are correctly aligned coaxially, hole **A** in the stop bullet **17** will be aligned with holes **A** in the guide bullet **20**, and so on. Alignment is facilitated by the indexing grooves **24A** and **24B** in the bullets **17** and **20**, respectively.

Figure 4 illustrates how the neural transplantation device, comprising the microinjector **1**, Hamilton syringe **3** and transplantation cannula **2**, and the bullet guide **16** are fitted to a Leksell stereotactic frame (Model A0260-02). When the stop bullet **17** and guide bullet **20** are mounted and coaxially aligned one above the other in a stereotactic frame, the transplantation cannula **2** can be precisely guided and inserted at a predetermined cerebral target.

In the initial stage prior to administration of a cell graft, the plunger **12** of the syringe **3** is in a foremost upward position and the syringe barrel **7** with attached guide nut **8** is in an unwound position inside the plunger guide **5**. When an injection is to be administered, the plunger driver **11** is rotate, thereby advancing the syringe plunger **12** in a downward vertical direction through the syringe barrel **7**. A specific volume of the cell suspension is subsequently aspirated and deposited through the port holes **15A** and **15B** of the transplantation cannula **2** at the target site. Prior to making a second injection and deposit of the cell suspension, the guide nut **8** is rotated 90° in a clockwise position thereby withdrawing the syringe **3** and cannula **2** in an upward vertical direction at a predetermined distance away from the first target site. Aspiration and delivery of the second volume of cell suspension is made by repeating the operation involving rotation of the plunger driver **11**. Sequential repetition of the steps involving rotation of the plunger driver **11**

and guide nut 8 to deliver the contents of the syringe 3 and reposition the cannula 2, respectively, allows several injections to be made per single trajectory thereby distributing the cells in a three-dimensional spiral array within the brain tissue.

Figures 5A and 5B provide front and top views, respectively, of a sequence of four injections, 3.0 mm apart, made in a single trajectory. The first injection delivers two graft deposits oriented opposite to each other and one at a slightly higher level than the other (solid balls). The cannula 2 is then withdrawn 3.0 mm in a stepwise fashion and rotated 90° clockwise and so that another two deposits can be made (solid balls). The process is repeated two more times until a total of 8 deposits are made per trajectory resulting in a three-dimensional spiral array.

Additional cell deposits at different trajectories are made by removing the microinjector 1 from its operative position, governed by the square grids,19 and 21, of the bullet guide 16, and then reinserting the transplantation cannula 2 of the microinjector 1 through another specified landmark of holes contained within the grids 19 and 21.

The following Examples illustrate the invention:

Example 1 - Animal Studies

Animals tolerated the transplant procedure well and all of them had surviving grafts 6 to 8 weeks after transplantation. Typically, two graft deposits were observed in the implanted striatum and each graft deposit corresponded to the upper and lower side holes of the transplant cannula (Figure 4). The cannula tract was clearly visible connecting the upper and lower graft and the deposits appear to be oriented in opposite directions.

Numerous TH immunoreactive cells and fibres were observed in the graft deposits and cannula tract (Figure 5). Fibres were also observed penetrating the host striatum for variable distances and overall the appearance of the grafts was comparable to animals grafted with a glass microcapillary in our laboratory (Mendez et al., *Brain Res.*, 778, pp. 194-204, 1997; Mendez et al., *J. Neurosci.*, 16(22), pp. 7216-7227, 1996). There was no evidence of significant trauma in the grafted area and no tissue disruption was observed in the cannula tract.

Example 2 - Clinical Studies

Eight patients enrolled in the Halifax Neural Transplantation Program received bilateral putaminal fetal VM tissue obtained from women undergoing elective abortions in the pregnancy termination unit of the Queen Elizabeth II Health Sciences Centre following strict guidelines of a protocol approved by the University and Hospital ethical review boards.

The surgical transplantation procedure was carried out in two stages in which each side was transplanted 4 to 6 weeks apart. Patients were admitted to hospital the night prior to surgery. On the day of surgery, patients were fitted with a Leksell stereotactic headframe under local anaesthesia. The stereotactic coordinates for targets in the putamen were calculated using T1-weighted MRI images and a computerized planning workstation (Surgiplan, Elekta AB, Stockholm, Sweden).

Transplantation was performed with the patient under local anaesthesia and sedation, using a combination of Midazolam (0.25 to 1.0 mg bolus doses) and Proprofol (10 to 20 mg bolus followed by infusion at 15 to 40 mg/kg/min). A burr-hole was placed at the level of the coronal suture and the transplantation cannula was inserted into four different

trajectories approximately 3.0 mm apart in the postcommissural putamen. A 50 µl Hamilton syringe, fitted with the microinjector, was used to load the 15 µl of cell suspension in the transplantation cannula. The cell suspension was prepared in the same manner as described in the animal experiments. The dead space in the cannula was filled first with medium solution in such a way that the 15 µl of cell suspension was only loaded in the most distal part of the cannula. The cell suspension was deposited along each of the four trajectories previously calculated on the patient's MRI scan. Four injections of approximately 2.5 µl each (eight deposits) were made in each trajectory for a total of 10 µl per trajectory. The injections were made 3.0 mm apart as the cannula was slowly withdrawn in a stepwise fashion and rotated 90° clockwise before each injection at a rate of approximately 1 µl/min (Figure 3). A wait of 2 minutes was observed between each injection and the cannula was completely withdrawn after 4 minutes from the last injection and the cannula was completely withdrawn after 4 minutes from the last injection. Approximately 4 million cells were deposited in each postcommissural putamen. Patients received 1 g of Ancef intravenously before the skin incision was made and three more doses of 1 g of Ancef intravenously every 8 hours post-operatively. Patients were discharged from the hospital 48 hours after surgery. Patients had an MRI which included T1, T2 and inversion recovery (TR 7000 msec, TE 60 msec and TI 400 msec) sequences in the axial, coronal and sagittal planes 24 hours after surgery to check for target accuracy and bleeding. MRI scans with gadolinium enhancement were also performed at 6 and 12 months after surgery to check for blood-brain-barrier breakdown.

PET scans were performed at the McConnell Brain Imaging Centre (Montreal Neurological Institute, McGill University) before and after the transplant procedure. Scans were performed on the Siemens ECAT HR+ Positron Emission

Tomograph in 3D mode, with a resolution of 5 mm FWHM in all directions at the centre of the field of view. Subjects received 5 mCi of [18F]DOPA (FD) as a bolus injection into the antecubital vein over 2 minutes. Their heads were immobilized within the aperture of the PET scanner by a form fitting vacuum device. One hour prior to the scan, subjects received carbidopa, 150 mg p.o. to prevent the peripheral breakdown of FD. On the day of the scan, patients did not receive anti-Parkinsonian medications and they did not eat breakfast prior to the scan. After the injection of FD, PET data was acquired for 90 minutes in 27 time-frames of varying durations.

A total of 16 transplant operations and 64 trajectories were performed on eight patients with the implantation cannula and microinjector system. The patients tolerated the surgical procedures well and there was no intra-operative or peri-operative complications. The brain MRI scans done 24 hours after surgery showed that the deposits were made in the desired targets in all cases (Figure 6) and there was no evidence of hemorrhage or tissue damage. The lesioned striatum (left) in Figure 6 shows the two graft deposits made by the transplant cannula. Note that the orientation of the upper and lower grafts (short arrows) corresponds to the side holes of the cannula. There is no evidence of significant trauma in the transplanted striatum and the grafts are well integrated in the host. The two grafts are connected by the cannula tract and contain TH-positive cells and fibres (long arrow).

The MRI scans with gadolinium enhancement done at 6 and 12 months after transplantation did not show any areas of enhancement which indicates no blood-brain-barrier breakdown.

Post-operative fluorodopa PET scans showed an increase in

fluorodopa uptake in the transplanted areas 6 and 12 months after transplantation (Figure 7). This increase in tracer uptake is an indication of graft survival.

MATERIAL AND METHODS

Implantation Cannula and Microinjector System

The cannula and microinjectory system (Figure 1) were designed to fit a 50 µl Hamilton syringe and could be adapted to be used with any stereotactic frame system. The Leksell stereotactic frame was used (Model A0260-02, Elekta AB, Stockholm Sweden) (Figure 2) and the only modification needed was to change the stop and guide to a custom made set with the appropriate diameter for the cannula (0.8 mm). The cannula was manufactured from a stainless steel 21-gauge needle (outer diameter 0.8 mm) and a length of 195 mm. A standard Luer lock was fitted to the proximal end. The cannula tip is rounded and polished to minimize trauma. The proximal end has two side holes (0.3 mm diameter). The first hole is 1.0 mm proximal to the tip and the second hold is 2.0 mm proximal to the first hole but oriented in the opposite direction (Figure 1).

The microinjector system was manufactured of acetal nylon and ionized aluminum. The microinjector consists of a threaded cylinder with an adapter for the syringe barrel placed distally and a plunger driver proximally (Figure 1). The syringe plunger is controlled by the plunger driver and can deliver the desired volume of cell suspension accurately.

Animal Experiments

Ten female Wistar rats (Charles River, St. Constant, Quebec, Canada) weighing 200 to 225 g were housed two animals per cage with food and water ad lib and allowed to

acclimatize for 7 days in the animal care facility prior to surgery or behavioural testing. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care and the University Council on Laboratory Animals. Rats received two stereotactic injections of 6-hydroxydopamine (6-OHDA) into the right ascending mesostriatal dopaminergic pathway under pentobarbital anaesthesia at the following coordinates: (1) 2.5 µl of 6-OHDA (3.6 µg 6-OHDA Hbr/µl in 0.2 µg/µl L-ascorbate 0.9% saline) AP -4.4, L 1.2, DV -7.8, tooth bar -2.4 and (2) 3 µl of 6-OHDA at AP -4.0, L 0.8, DV -8.0, tooth bar +3.4. The injection rate was approximately 1 µl/min. And the cannula was left in place for an additional 4 minutes before slowly being retracted. Following a two-week recovery period in the animal care facility, animals were given an amphetamine challenge (5 mg/kg i.p.) And their rotational scores collected over a 90-minute period. Only animals exhibiting a mean ipsilateral rotation score of nine or more full body turns/minute were included in the study.

The lesioned rats were transplanted using the clinical cannula and microinjector system. In brief, cell suspensions were prepared from VM of 14-day old rat fetuses and injected stereotactically in the host brains of 6-OHDA lesioned animals. Cell suspensions of fetal VM tissue were prepared by the following procedure. The tissue was incubated in 0.1% trypsin/0.05% DNase/DMEM (Trypsin: Worthington; DNase: Sigma DN-25) at 37°C for 20 minutes , then rinsed four times in 0.05% DNase/DMEM. The tissue was then mechanically dissociated into a "chunky" cell suspension. A final cell concentration of approximately 200,000 cells/µl was used with viability of 98% as determined by the trypan blue dye exclusion method. A total of 500,000 cells (2.5 µl) were implanted into the dopamine-depleted striatum.

Six to eight weeks after transplantation, rats were anaesthetized with an overdose of pentobarbital and perfused transcardially with 100 ml of 0.1 M phosphate buffer, followed by 250 ml of 4% freshly-depolymerized paraformaldehyde in 0.1 M phosphate buffer over 15 minutes. Brains were removed and post-fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer before being stored in phosphate-buffered saline containing 30% sucrose for 24 hours. Coronal sections 40 µm thick were cut on a freezing microtome and collected serially in 0.1 M phosphate buffer. Sections were processed for tyrosine hydroxylase (TH) immunohistochemistry using a primary rabbit anti-TH antiserum (1:2,500 Pel Freeze) and the ABC-kit (Vector, Dimension Laboratories).

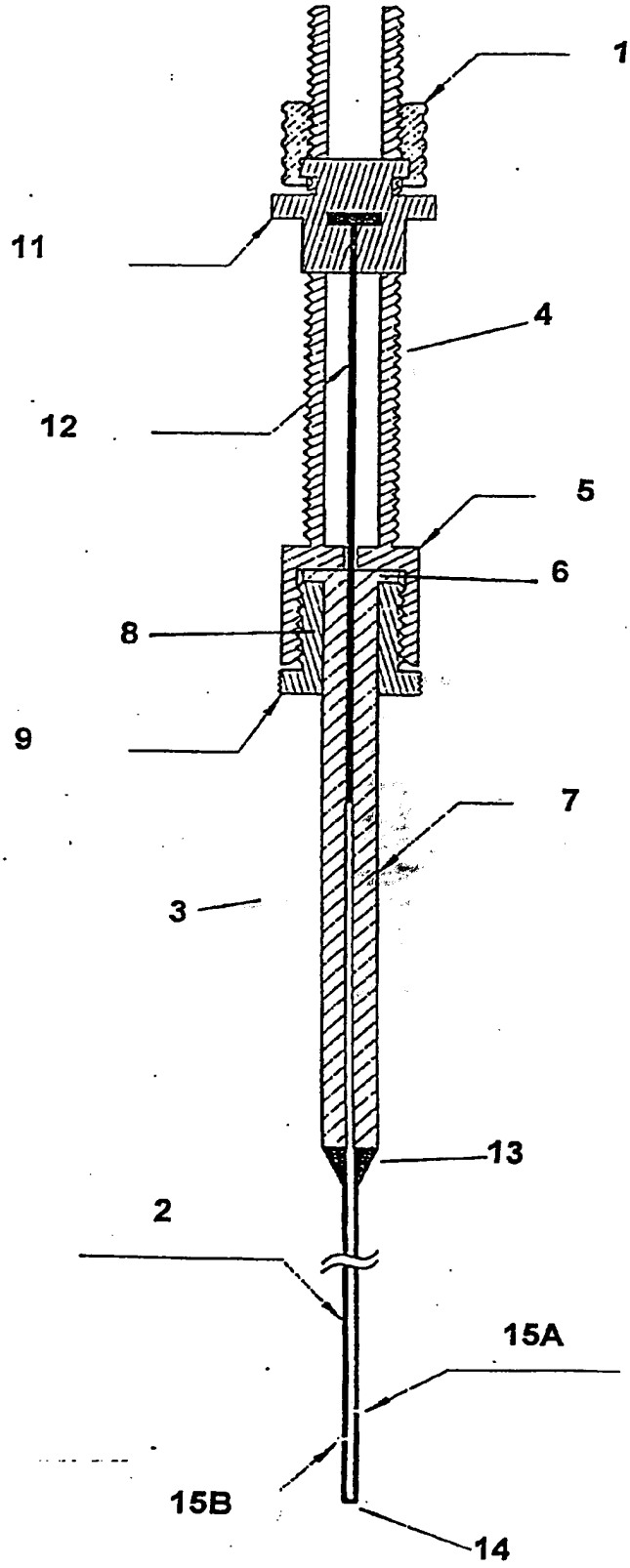


Figure 1

Figure 2

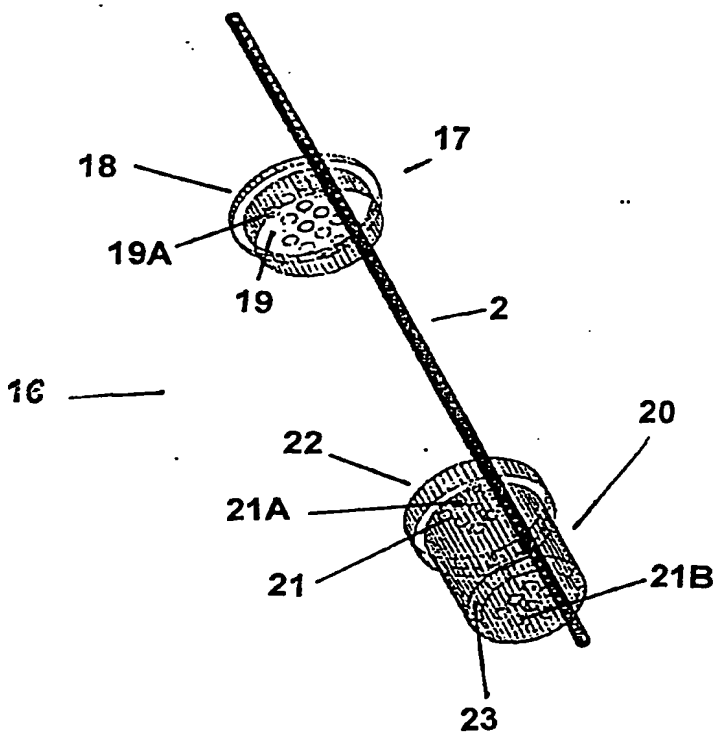
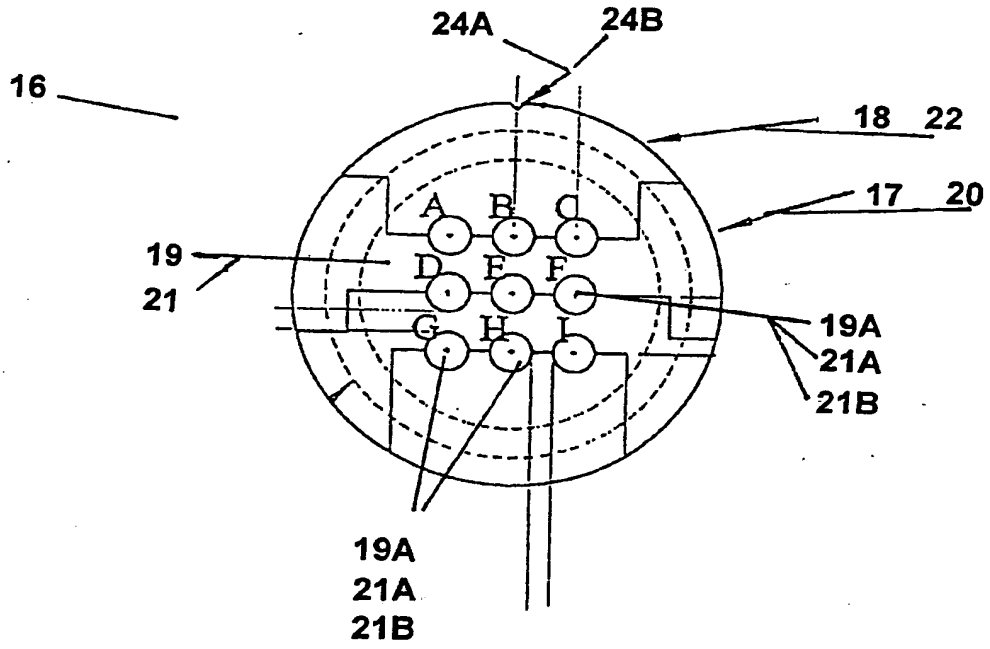


Figure 3



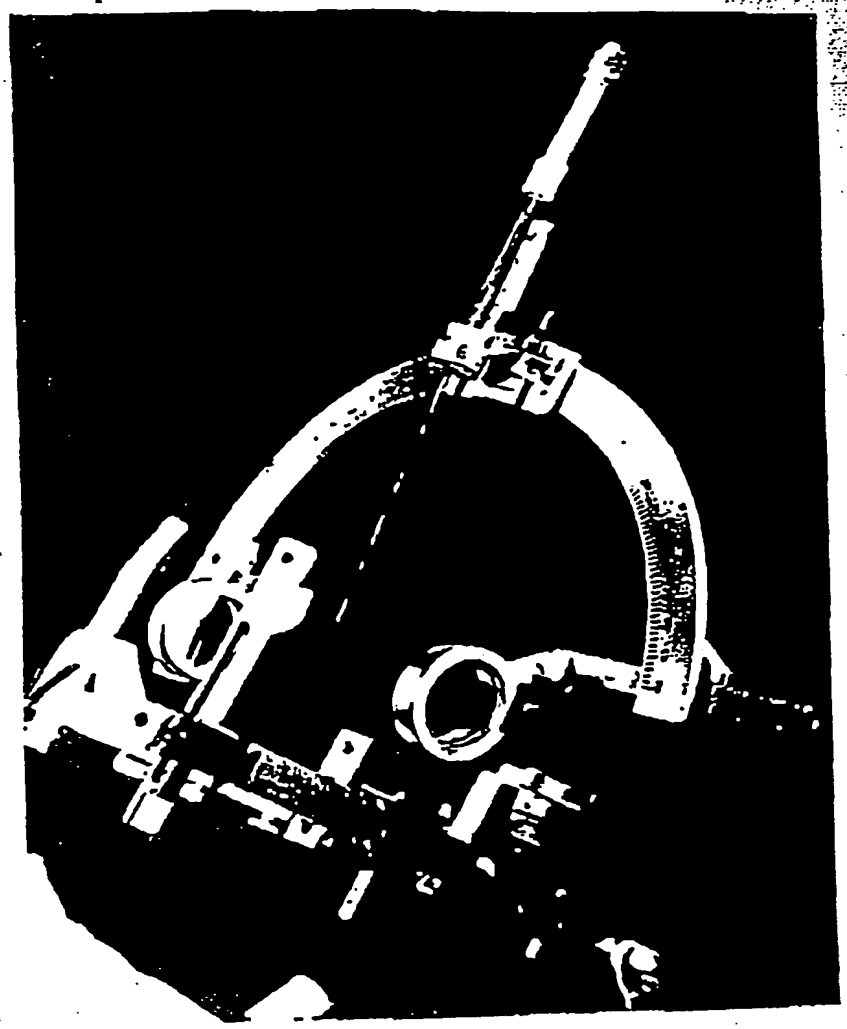


Figure 4

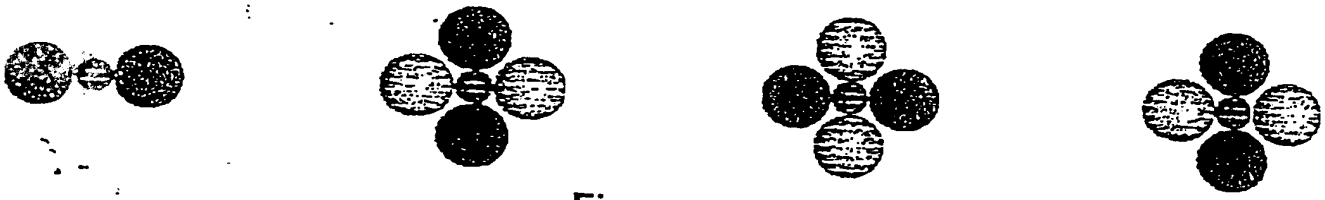


Figure 5B

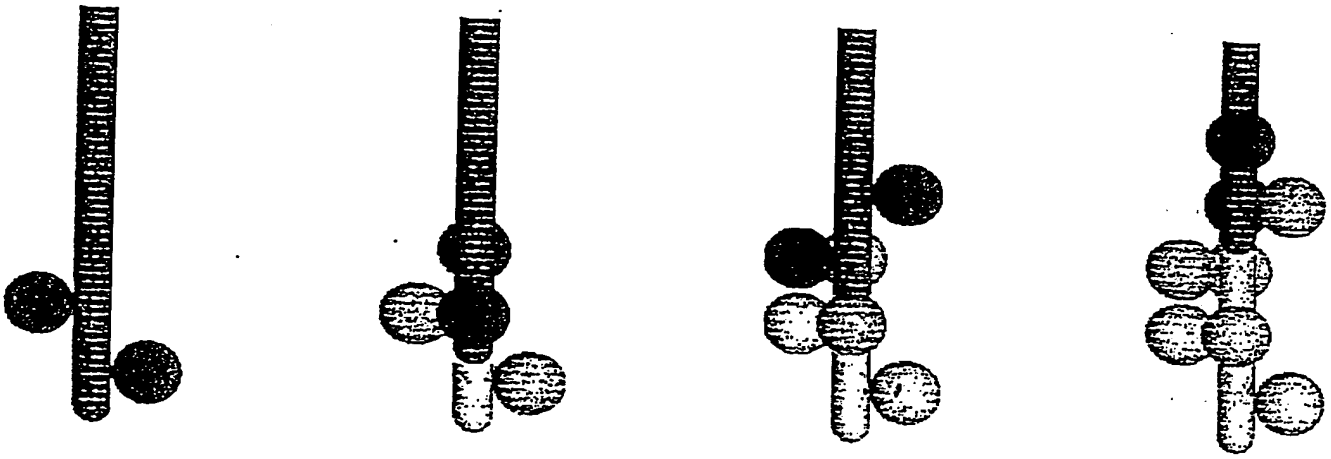


Figure 5A

Figure 6

Figure 7

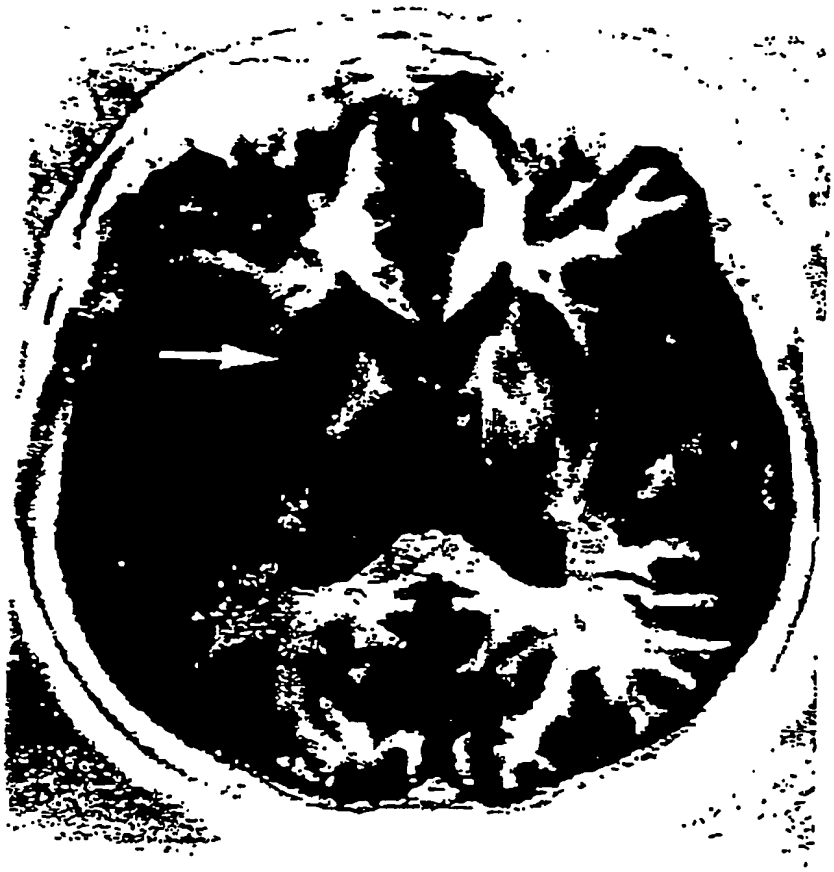


Figure 8

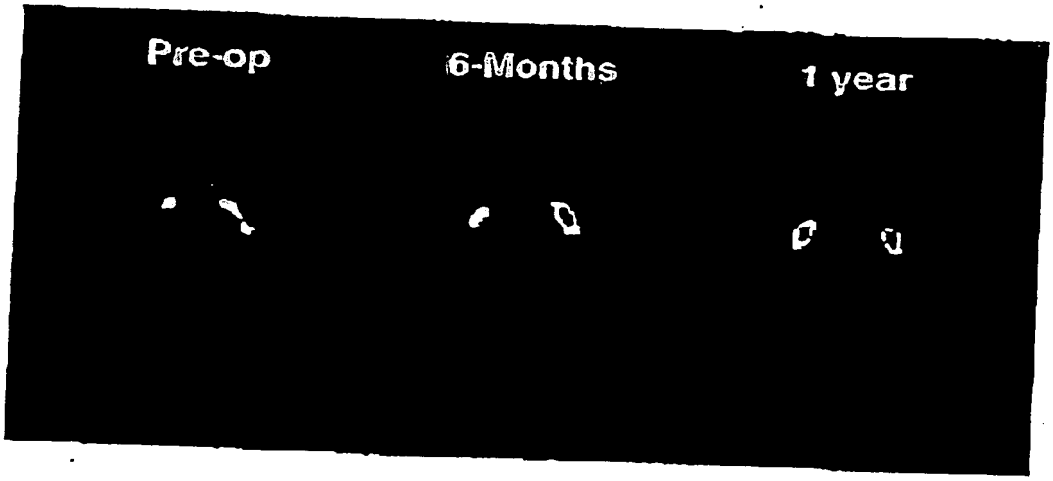


Figure 9